

REMARKS UNDER 37 CFR § 1.111

Formal Matters

Claims 1-45 are pending after entry of the amendments set forth herein.

Claims 1-45 were examined. Claims 1-33 and 45 were rejected. Claims 8 and 9 were objected to. Claims 34-44 are withdrawn from consideration.

Applicants respectfully request reconsideration of the application in view of the amendments and remarks made herein.

No new matter has been added.

Amendments to the Specification

Applicants have amended the specification to capitalize two trademarks, as per the Examiner's request. This clerical amendment adds no new matter.

Amendments to the Claims

Applicants have amended claims 1, 6, 7, 8, 10 - 14, 23, 25 - 30, 32 - 33 and 45 more particularly to point out and distinctly claim applicants' invention. Support for these amendments is found in the claims as originally filed, as well as in the specification at, for example, page 19, lines 16-22 (total ionic salt concentrations); and page 28, line 30 to page 29, line 2 (increase "enhance" discrimination).

Objections to Claims and Rejection of Claims Under 35 U.S.C. §112, Second Paragraph

Applicants respectfully submit that the amendments obviate the Examiner's objection to claims 8 and 9 and the rejections of claims 1 - 33 under 35 U.S.C. § 112, second paragraph, and respectfully request that the objection and rejections be withdrawn.

Rejections Under 35 U.S.C. § 102(e) and § 102(a) Have Been Obviated Or Are in Error and Should Be Withdrawn

The Examiner rejects claims 1 - 33 and 45 -- all of the claims currently under examination -- under 35 U.S.C. §§ 102(e) and 102(a) as anticipated by Cronin, U.S. Pat. No. 6,027,880 (Cronin).

Applicants respectfully disagree.

As clarified by amendment herein, applicants' claimed methods require that nucleic acid duplexes be formed in a hybridization reaction "in the presence of *a specific association enhancer* under *conditions suitable for accelerated association of specific duplexes.*"¹

Association enhancers are "agents that accelerate the rate at which complementary single-stranded nucleic acid molecules form base-paired double-stranded duplex molecules...." Specification p. 6, lines 22 - 24.

CTAB is one such association enhancing agent: "Pontius has reported . . . that cationic detergents like cetyltrimethylammonium bromide (CTAB) can . . . accelerate the association rate of DNA:DNA duplexes..." Specification page 4, lines 28 - p. 5, line 1. "Publications by other researchers have shown that CTAB and other CTAB-like detergents can accelerate formation of RNA:RNA molecules." Specification p. 5, lines 8 - 9.

Applicants have discovered that certain of these accelerating agents, under certain reaction conditions, "can also greatly increase the specificity or selectivity of formation of completely matched

duplexes over mismatched ones.... " Specification p. 6, lines 26 - 27. That is, applicants have discovered that "certain association enhancers, herein termed 'specific association enhancers', act in certain hybridization reactions both to increase the reaction rate and improve the specificity of the hybridization." Specification, p. 8, lines 21 - 23 (emphasis added).

"An advantage of the invention is that the methods provide for accelerated association of single-stranded nucleic acid molecules in a manner *that also* provides for extreme specificity, e.g., the method provides for rapid detection of single nucleotide differences between two nucleic acid sequences (e.g., between an RNA sequence and a DNA sequence)." Specification p. 6, lines 8 - 11 (emphasis added).

The Examiner contends that "Cronin et al disclose the use in hybridization buffer of agents that 'improve discrimination between perfectly matched targets and single-base mismatches,' including tetramethylammonium chloride and betaine (col 59, line 61 - col 60, line 6), and exemplify the use of one such an agent, cetyltrimethylammonium bromide (CTAB). . . ."²

First, a disclosure of an agent that improves specificity of hybridization is not a disclosure of an agent that both improves specificity and accelerates the hybridization reaction -- that is, a disclosure of a specific association enhancer -- as required by applicants' claims.

Second, the section of the Cronin patent cited by the Examiner, dealing with alleged specificity enhancers, does not in fact exemplify the use of CTAB as a specificity enhancing reagent. Nor is CTAB mentioned, described, or taught anywhere else in the Cronin patent to be an agent capable of enhancing specificity of hybridization.

Nor could it be, given the elevated salt concentration used by Cronin in hybridizations containing CTAB. In Cronin's single reference to CTAB,

[l]abeled, fragmented PCR product . . . was diluted 10 to 25 fold into 5X SSPE (750 mM NaCl, 50 mM NaPhosphate, 5 mM EDTA, pH 7.4) and 1 mM cetyltrimethylammonium bromide (CTAB, Sigma) and used directly in hybridizations.... Targets were hybridized separately in 1 - 3 ml

¹ Claim 1, as amended herein. Claim 45, as amended, similarly recites "combining . . . in the presence of an a *specific association enhancer*, said combining being *under conditions suitable for accelerated association* of the first and second molecules *in a specific nucleic acid duplex*" (emphasis added).

² Office action, item 10, pages 5 - 6.

of 5X SSPE with 10 mM CTAB at 30°C for 30 minutes. The arrays were washed briefly (1 - 5 minutes) at 25°C - 30°C with 5X SSPE and 0.01% SDS prior to imaging."

Cronin, column 47, lines 45 - 60.

Applicants have discovered that increase in the "ionic salt concentration [e.g., NaCl concentration] to at least about 0.7M" "disrupt[s] the association of the specific association enhancer and nucleic acid duplex." Specification p. 22, lines 18 - 21. Indeed, "[w]here the specific association enhancer is a cationic detergent, such as CTAB or a CTAB-like cationic detergent, the invention further presents, in another aspect, methods to remove the specific association enhancer from the hybridization reaction," Specification, lines 18 - 20, by increasing the salt concentration "until said hybridization reaction comprises greater than 0.7M total ionic salt concentration," claim 32, as herein amended.

Cronin could not possibly have seen, let alone described, use of CTAB as a specificity enhancer using salt concentrations that the present applicants have discovered dissociate the agent from the nucleic acids.

Third, Cronin neither mentions, describes, nor teaches that either betaine or TMAC, which are alleged by the Office Action to enhance specificity, can serve as hybridization accelerants. Indeed, the reported four-fold lower signal in the presence of betaine (Cronin, column 60, lines 3 - 6) would argue that betaine no more accelerates the hybridization than does low salt concentration, which is well known to increase specificity and decrease signal.

"A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." MPEP § 2131, quoting *Verdegaal Bros. V. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987).

Applicants respectfully submit that the Cronin patent does not describe, either expressly or inherently, a method of enhancing discrimination among a plurality of nucleic acid targets (claim 1), or of increasing the specific association rate of a pair of single-stranded nucleic acid molecules (claim 45), by addition to the hybridization reaction of a specific association enhancer under conditions in which it both accelerates the hybridization and increases the specificity thereof.

Accordingly, applicants respectfully submit that the Cronin patent does not anticipate claims 1 and 45, and claims dependent therefrom, and that the rejection should be withdrawn.

Conclusion

Applicant submits that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number STAN-202.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: Oct 31, 2003

By: Carol L. Francis

Carol L. Francis
Registration No. 36,513

BOZICEVIC, FIELD & FRANCIS LLP
200 Middlefield Road, Suite 200
Menlo Park, CA 94025
Telephone: (650) 327-3400
Facsimile: (650) 327-3231